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APPLICANTS:	Nathan H. Sloane		
SERIAL NUMBER:	08/986,606	EXAMINER:	David Lukton
FILING DATE:	December 8, 1997	ART UNIT:	1653
FOR:	THE USE OF THE ACTIVATED N-TERMINAL SIXTEEN AMINO ACID PEPTIDE OF THE ANTINEOPLASTIC PROTEIN (ANUP) AS A PHARMACOLOGICALLY ACTIVE ANTI-TUMOR AGENT		

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

July 2, 2004
Boston, Massachusetts

STATEMENT IN SUPPORT OF SUBSTITUTE SPECIFICATION

Pursuant to 37 CFR 1.125(b)(1), I hereby state that the content of the Second Substitute Specification filed herewith and the specification filed on December 8, 1997 are the same. Applicant hereby submits this Second Substitute Specification under the provisions of 37 CFR 1.125 (b) and (c) in response to the non-final Office Action mailed on January 2, 2004 in the above-identified application. In the Second Substitute Specification submitted herewith, Applicant has amended the specification in accordance with the preferred arrangement of the specification. In particular, Applicant has re-arranged certain sections of the as-filed specification, but Applicant has not added any additional information. For example, Applicant has deleted the section entitled "ABSTRACT" on page 2 of the as-filed specification and added a portion of this section as the amended Abstract, which has been presented on a separate sheet of paper from any other part of the specification. The amended Abstract, presented on page 6 of the substitute specification, corresponds to the information presented on page 2, lines 2-4 of the as-filed specification. Applicant has also amended the specification to remove the bibliographic information originally presented on page 1 of the as-filed specification. In addition, Applicant has also added a brief section entitled "BACKGROUND OF THE INVENTION", which

corresponds to the information originally presented at page 3, lines 14-17 and at page 2, lines 7-9 of the as-filed specification. Under the section "DETAILED DESCRIPTION OF THE INVENTION", Applicant has added a paragraph at page 2, line 23 through page 3, line 3 of the substitute specification, which is identical to the information presented on page 2, line 37 through page 3, line 5 of the as-filed specification. Applicant has also added a paragraph at page 2, lines 18-28 of the substitute specification, which corresponds to information originally presented on page 2, lines 2-14 of the as-filed specification. Finally, Applicant has amended the specification, *e.g.*, at pages 4-5 of the as-filed specification to correct typographical errors. All references to "ug" and "ul" as units of measurement on pages 4-5 of the as-filed specification have been replaced with the appropriate scientific abbreviations, "μg" and "μl". In addition, the term "gestamycin" at page 4, line 8 has been amended to correct the spelling of "gentamycin." Accordingly, this submission includes no new matter.

Pursuant to 37 C.F.R. 1.125(b)(2) and 1.125(c), Applicant submits herewith a clean version of the Second Substitute Specification, including all amendments to the specification, and a marked-up version indicating all amendments to the specification.

Applicants believe that no additional fees are required for the filing of the present submission. However, the Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment, to Deposit Account No. 50-0311, Ref. No. 21578-013.

Respectfully submitted,

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THE USE OF THE ACTIVATED N-TERMINAL SIXTEEN AMINO ACID PEPTIDE OF THE ANTINEOPLASTIC PROTEIN (ANUP) AS A PHARMACOLOGICALLY ACTIVE ANTI-TUMOR AGENT

FIELD OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent.

BACKGROUND OF THE INVENTION

The Antineoplastic Protein (ANUP) kills tumor cells. The protein (ANUP) in the purified state has been implicated in regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice.

SUMMARY OF THE INVENTION

The present invention describes the pharmacologically active anti-tumor activity of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP).

The 16 amino acid peptide is approximately one-half as active as the protein on a molar basis utilizing the human breast tumor cell line (MDA 231). However, only about one-tenth of the weight of the peptide is required when compared to the amount of protein for equivalent activity against the human breast tumor cell line. Both the protein and the peptide exert their action by killing tumor cells (apoptosis) since electron microscopy studies showed complete degradation of the cells (Struve et al. Cancer Res. Therapy and Control (1990) 1: pp 225-230).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a

pharmacologically active antitumor agent. The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9} M).

DESCRIPTION OF THE PREFERRED EMBODIMENT

The 16 Amino Acid Peptide

The synthetic hexadeca peptide (16 L-amino acids) has the following sequence:

1.	Pyroglu		9.	Glu	E
2.	Leu	L	10.	Pro	P
3.	Lys	K	11.	Met	M
4.	Cys	C	12.	Thr	T
5.	Tyr	Y	13.	Ser	S
6.	Thr	T	14.	Ala	A
7.	Cys	C	15.	Ala	A
8.	Lys	K	16.	Cys	C (SEQ ID NO: 1)

The peptide was synthesized by Research Genetics Inc., in Huntsville, AL 35801; the peptide was pure as shown by HPLC (high performance liquid chromatography) and the molecular weight was check by mass spectrometry (MS).

The 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) is a highly active pharmacologically antitumor agent. The 16 amino acid peptide is about 50% as active as antitumor agent compared to the antitumor active as the protein (ANUP) per se when tested as a tumor killer agent (in vitro) utilizing human breast tumor cell line (MDA 231). The protein (ANUP) in the purified state also shows regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice (Sloane, Davis Tumor Targeting (1996) 2, pp 322-326). The nonapeptide is about 10% as active compared to the antineoplastic protein (ANUP) in the human breast tumor cell line in vitro assay system. Both peptides, the 9 amino acid peptide and the 16 amino acid peptide require presence of the detergent sodium dodecyl sulfate to activate the peptides for full pharmacological antitumor activity.

EXAMPLES

Example 1: The pharmacological anti-tumor activity of the 16 amino acid peptide (P_{16})

The antitumor activity of the peptide (P_{16}) was assayed against the human breast tumor cell line (MDA 231) and its activity was compare to the in vitro antitumor effect of the "pure" protein (ANUP).

The assay for the pharmacological antitumor activities were performed as follows utilizing 96 well plates --

20,300 - 30,000 human breast tumor cells in L-15 medium (200 μl) containing 2.5 % fetal calf serum and 100 μg gentamycin per ml (complete medium) were incubated at 37° in air for 120 hours; after this incubation period 50 μl of serially diluted P_{16} and ANUP were added to each well. The serial dilutions were prepared as follows: 2 mg each (the P_{16} and ANUP) were dissolved in 2 ml of complete medium containing 0.05% sodium dodecyl sulfate (SDS). The solutions were diluted in complete medium containing 0.5% SDS to a concentration of 350 μg per ml.

Dilution plates were prepared as follows:

100 μl of complete medium were added to each well and 50 μl of diluted P_{16} and ANUP were added to each well in row A thus 1.3 dilution was accomplished; 50 μl were serially diluted in the 100 μl of medium in rows B through H. Thus the range of concentrations were from 6 μg to 2 mg when 50 μl each dilution series were added to the 200 μl of the complete medium containing the MDA cells. The plates were incubated for an additional 96-120 hours. The medium was poured off and after a 90-minute incubation with 50 μl neutral red dye (0.5 ml neutral red (0.25% ethanol (0.6 ml) diluted 5.5 saline - 0.16 mm HCl) the cells were washed twice with PBS (phosphate buffer saline) at room temperature. The concentration of living cells (since only living cells absorb the dye) was determined after adding 100 μl lysing buffer (50% ethanol in 0.05 m NaH_2PO_4) the concentration of neutral red released in each well was determined using a Dynetech plate reader set at 550 mm. A unit of activity was defined as the concentration of ANUP and P_{16} for 50% killing.

Under these assay conditions the 50% end points were as follows:

ANUP 0.1 μg/well = 1.25×10^{-8} M

P_{16} 0.0 μg/well = 2.2×10^{-8} M

Thus, P_{16} is about 50% as active as ANUP on a molar basis; whereas on a weight basis only one tenth of the peptide weight is equal in activity 10 times the weight of the protein (ANUP).

In the absence of SDS neither the peptide nor the protein showed any antitumor activity. Thus the detergent is probably necessary to form the correct geometrical shape for activity as described by Sloane and Davis Tumor Targeting (1996) 2, 322-326. The data utilizing P_{16} as an antitumor agent against the human breast tumor cell line (MDA 231) are as follows:

	Fraction of the Activity relative to ANUP
P_{16} no SDS	± no Activity
P_{16} + 0.005% SDS	0.04
P_{16} + 0.02% SDS	0.50
P_{16} + 0.05% SDS	0.50